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Light and electron microscopy of Paranella diplodae N. SP. (Polyopisthocotylea;Microcotylidae; Monogenea) from some Red Sea Teleost fishes in Egypt

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The present study deals with a monogenean parasite infecting, some marine fish through light and scan electron microscopy. It revealed that the percentage of infection was 48% (14 out of 50 fish), 28% (14 out of 50 fish), 22% (11 out of 50 fish) and 16% (8 out of 50 fish) in *Diplodus noct, Gerres oyena, Lethrinus elongates* and *Siganus revulatus,* respectively. The present work recorded *Paranaella diplodae* (Polyopisthocotylea; Microcotylidae; Monogenea) as a new species collected from the investigated fish gills. They are lanculate flukes, the haptor is not distinguished from the body proper approximately 1/3 of the whole body length. The surface topography of the parasite bears small pits and conspicuous transverse folds and richly supplied with papillae-like unicellate sensory ending. The opisthohaptor is typical of *Microcotylidae*. The clamp structure and the haptoral tegument are similar to the rest of the body.

Monogenea is the most common and abundant ectoparasitic flukes of fish, with greater diversity of species widely distributed in the tropics than in the moderate region of the world (Rohde, 1982; Ramasamy *et al*., 1995). Most of them are parasites mainly on the gills of fish and may be exhibit both host and site specificity (Cohen *et al*., 2001) causing economic losses and become pathogenic in some cases (Paperna *et al.,* 1984).

Scanning electron microscopy provide resolution at high magnification, which has more recently been exploited in helminthology to elucidate and describe the specialized organs of attachment, the position of the oral and genital apertures which are considered as additional diagnostic and taxonomic characters (Ramasamy and Hanna, 1985, 1986a, b, 1989; Ramasamy *et al*., 1986, 1987,1995; Ramasamy and Bhuvan-eswar, 1993; Ramasamy and Brennean 2000).

Most studies of monogenean parasites in Egypt were carried freshwater fishes (El-Naggar and Serag, 1986, 1987; Khidr, 1995, 1996, 1997; El-Naggar *et al*., 2000,2001; El-Naggar & El-Abassy, 2003). Little attention has paid to parasites of marine fish, especially monogenetic trematodes, except that carried by Paperna (1972a,b,c) and Bayoumy, (2003). Hence, this work aims to study the presence of monogenean parasites of some economically important marine Red Sea fish by light and scan electron microscopy.

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Materials and Methods

A total of 200 alive specimens of teleost fishes belonging to four families and species according to Randall, (1983) as follow; Sparidae: *Diplodus noct*, Cuvier and Valenciennes ,1830, Lethrinidae: *Lethrinus elongatus*, Cuvier and Valenciennes,1830, Gerreidae: *Gerres oyena*, Frosskäl, 1775 and Siganidae: *Siganus revulatus*, Frosskäl,1775, caught from the Red Sea at Balayium area, South Sinai. The fish specimens brought to the laboratory in aerated tanks to examine their gills for the presence of monogenean parasites.

Fish anaesthetized and their gills removed in small Petri dishes containing filtered seawater to get rid of excess mucus. The gills were examined under dissecting binocular microscope and the parasites were removed by using long tipped Pasteur pipette to small Petri dish containing also filtered sea water to remove the excess mucous gaining from the flukes.

For light microscopy, the isolated parasites were fixed in 10% formalin and washed with distilled water to remove excess fixative. For staining, acetic acid alum carmine was used according to (Carleton 1967) for 10-30 minutes. Dehydration was maintained by passing in

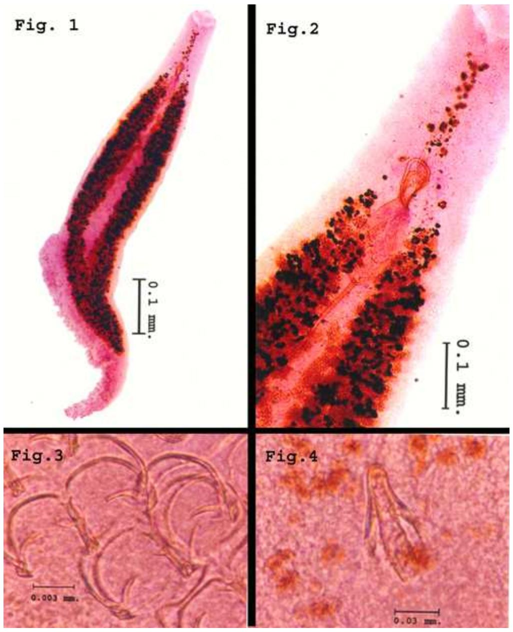


Fig. (1): A light photomicrograph of whole mounted *Paranaella diplodae* n. sp. Note: the lanculate shape of the fluke.
Fig. (2): A light photomicrograph of the anterior portion of *P. diplodae* n. sp. showing the reprod-uctive system structure.
Fig. (3): A light photomicrograph showing the clamp structure of *P. diplodae* n. sp.
Fig. (4): A light photomicrograph viewing the shape of the genital atrium and the con structure of *P. diplodae* n. sp.

ascending series of ethyl alcohol, cleared in clove oil and then mounted in Canada balsam. For scanning electron microscopy (SEM), living specimens were kept for 30 minutes in the refrigerator before fixation in 4% aqueous gluteraldehyde solution at 4°C for 48 hrs. (Halton, 1979). The specimens were then washed thoroughly with cacodylate buffer and post fixed for 4 hrs. with aqueous osmium tetroxide ($Os O_4$), dehydrated through acetone, dried in Polaron Equipmement, E300 critical point drying apparatus using liquid CO_2, mounted on aluminum stub with double phase sticker. The specimens then coated with gold-palladium in an E5000 sputter coating unit (Polaron Equipment) coating unit and examined in a Joel SEM T330 scan-electron microscope operating at 20 Kev. The mean was out of 15 specimens ± S.D.

Results

Type host. *Diplodus noct,* 48% (24 out of 50 fish examined).

Other hosts. *Gerres oyena,* 28% (14 out of 50 fish examined), *Lethrinus elongatus,* 22% (11 of 50 fish examined) and *Siganus revulatus*, 16% (8 out of 50 fish examined)

Site of infection. Gills

Locality: Balyium area, South Saini, Egypt.

Description. (Based on 15 whole mounted specimens) These are lanculate long flukes, tapering anteriorly with slight constriction in region of vaginal pore with a total body length 0.70–1.20 (0.75±0.26) mm.; the anterior body length 0.924–1.51 (0.55±0.2) mm.; the opistho-haptor length 0.27 -0.46 (0.35±0.11) mm. and the maximum body width 0.09–0.17 (0.12 ±0.02)mm.,(Fig.1,5). The wide mouth is sub terminal in position with an irregular slit,(Fig.7). There are unarmed broad buccal sucker provided with a septum measuring 0.39–0.05 (0.046 ±0.002) x 0.032–0.045 (0.041±0.005) mm., (Fig. 8). The buccal cavity leads to a muscular oval pharynx, measuring 0.021–0.027 (0.024±0.001) x 0.018 – 0.024 (0.021±0.001) mm. (Fig. 2). The esophagus is thin-walled, ramified and bifurcating interiorly to the genital atrium. The intestinal crura are not united and confluent posteriorly to the haptor. The outer diverticula intended into haptor and never united posteroirely. Pigmented granules scattered through the regions occupied by vitellaria and a few groups of them are located in the region between the pharynx and the genital atrium,(Fig. 2). The whole tegument of the fluke bears small pits and conspicuous transverse folds. It richly supplied with papillae-like uniciliated sensory ending,(Fig.9,10). Testes are numerous, being 7-12 (10) in number, situated in post-ovarian intercaecal field and extended to the haptor. The genital atrium (Fig. 4) is immediately posterior to the gut bifurcation without spins and measures 0.04-0.07 (0.061 ± 0.02) mm. long while its length is 0.051 – 0.075 (0.065 ± 0.05) mm. The ovary is pre-testicular and is located in the middle part of the worm body. It has an irregular tubular appearance with long coiled posterior part, while folded back on itself interiorly. The single vagina opens in the mid-dorsal region with an inverted Y-shaped vaginal duct, (Fig. 2). The typical *Microcotylidae* (Yamaguti, 1963) clamp structure is arranged in two sub-equal rows; being 25-37 (32) and 22-33 (28) in the right and the left sides, respectively, (Fig. 1,3,6). Their internal margins created depending on the state of contraction weather opened or closed. The haptoral tegument is similar to the rest of the body, different only in the absence of microvillus-like projection. All clamp shapes are

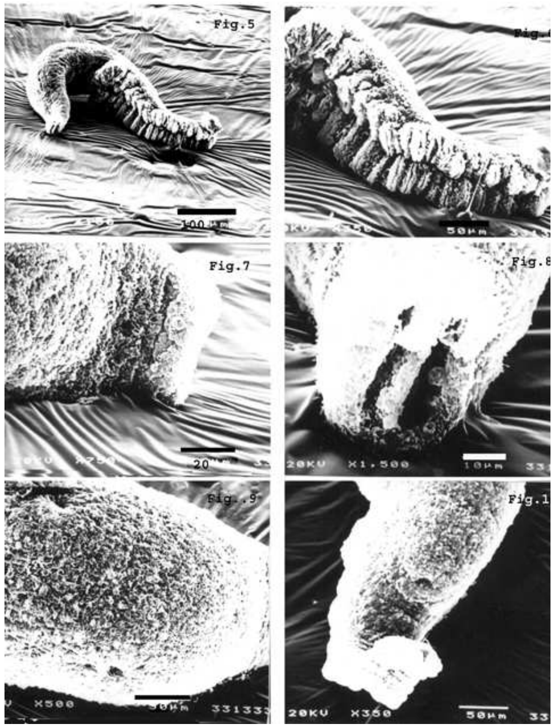


Fig. (5): Scanning electron micrograph of *P. diplodae* n. sp. the general parasite morphology
Fig. (6): Scanning electron micrograph of the ventral surface of *P. diplodae* n. sp. Note: the microcotylide clamp shape.
Fig. (7): Scanning electron micrograph of the ventral surface of the anterior portion of *P. diplodae* n. sp. Note: the sub-terminal position of the mouth and genital atrium.
Fig. (8): Scanning electron micrograph of the anterior view of *P. diplodae* n. sp. Note: the septum between the buccal sucker.
Fig. (9): Scanning electron micrograph of the dorsal surface of the anterior part of *P. diplodae* n. sp. Note: the conspicuous transverse folds and the large number different sizes of pits
Fig. (10): Scanning electron micrograph of the opisthohaptor in dorsal view of *P. diplodae* n. sp. Note: the similarity of the opsithohaptoral tegument to the rest of the body.

similar, but are slightly differ in size, which is clearly visible in the middle clamps where they attain lager size than that of the anterior and posterior ones. The anterior clamp measures 0.05 –0.065 x 0.019–0.024 (0.055±0.003 x 0.021± 0.002) mm.; the median one measures 0.042 – 0.061 x 0.016–0.021(0.047±0.003 x 0.018± 0.002) mm.; while the posterior one is 0.038– 0.056 x 0.014 – 0.019(0.045±0.005 x 0.017± 0.002) mm . The clamp structure measurements is as follow: the median bar length is 0.035– 0.047 (0.042±0.002) mm. and the longitudinal bar length is 0.052–0.073 (0.062±0.005) mm.(Fig. 3).

Discussion

As proposed by Kohn *et al*. (2000), the genus *Paranaella* (*Microcotylidae*) possesses the hereby-presented characteristics: body lanculate, haptor sub-terminal with numerous clamps armed into sub-equal rows. Clamps are of microcotyle type, similar in shape. Hooks are absent. Paired buccal suckers unarmed, with septum. Intestinal crura ramified, co-extensive with vitellaria, extending into haptor. Testes are numerous post-ovarian. Cirrus not differentiated. Genital atrium a muscular ring with numerous long, straight spins deposed into two levels of concentric row. Ovary tubular, anteriorly folded back on itself.

The parasite under discussion was identified according to Kohn *et al.* (2000) and Yamaguti, (1963) were has most of the generic features of the genus *Paranaella*, except there is no spins observed in the genital atrium. The present monogenean flukes are greatly similar to *Paranaella luquei*, Kohn *et al*. (2000), in most morphological features. While, the opisthohaptor of the present material does not distinguished from the rest of the body proper, triangular in shape, its lateral margins well-developed and the clamp structure is typical of Microctylidae (Yamaguti, 1963). However, the opisthohaptor of *Paranaella luquei*, Kohn *et al*. (2000), distinguished from the body proper. On the other hand, there is no significance difference between the measurements of the opisthohaptoral clamps of the present parasite and that of recorded by Kohn *et al*. 2000, (*Paranaella luquei*). Despite the fact that, the relative size of the opisthohaptor of the parasite under discussion is approximately 1/3 of the total body size, thus, it is relatively larger than that measured by Cohen *et al.* (2001) for *Paranaella luquei* (approximately 1/6 of the whole body length). In addition, the number of clamps of the present parasite is less than that of *Paranaella luquei* (right side 32 vs 41 and left side 28 Vs 35, respectively).

Ultrastructure of the tegument of monogenean by SEM had revealed that the topography of the surface shows variations in the surface architecture, most of them with functional significance (Cohen *et al.,* 2001). The present material surface supplied with papillae like uniciliate sensory ending and there are conspicuous transverse folds. These structures are characteristics of the tegument of the majority of the monogenea species studied, including *D. aclidophora merlangi* (Halton, 1979), *Microcotyle labracis* (Oliver, 1981),

Heterapta chorinemi (Ramasamy and Hanna, 1986b).

The folds of papillae like structures present in the tegument increase the surface area suggesting metabolic exchange (Oliver, 1981), and may help in respiratory gaseous exchange and osmoregulation (Halton, 1978, 1979), where the previous author suggested that the occurrence of microvelli and the absorptive function of the tegument provide evidence of a close phylogenetic relationship between Monogenea and cestodes. The significance of this high folding surface tegument increases with the secretory surface area. This also, increases the efficiency of the tegumental surface in protection from the effect of the surrounding media (Khidr, 1997). Therefore, from the all previously discussed points, the parasite under discussion has all the generic diagnosis of the genus *Paranaella* but it differs from *P. luquie* in the absence of genital spines and the number of opsithohaptoral clamps.

Accordingly, the present monogenean parasite suggested being a new one and the name *Paranaella diplodae* derived based on the type of its new host, *Diplodus noct*.

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فـحـص بالمجهـر الضـوئـي و المـاسـح الألـكـتـرونـى لـطـفيلى جـديـد "بارانيللا ديبلودى"(بولى أوبيثسوكوتيليا؛ ميكروكوتيليدى؛مونوچينيا) من بعض أسماك الـبـحـر الأحـمـر الـعـظـمـيه- بـمصر

تم فحص مائتي عينة من بعض اسماك البحر الأحمر العظمية بمنطقة بلاعيم (جنوب سيناء) لدراسة الطفيليات وحيدة الجيل التي تصيب خياشيم هذه الأسماك باستخدام المجهر الضوئي والمجهر الألكتروني الماسح وكذلك دراسة نسب الأصابة بهذا الطفيلي. سجلت الدراسة لأول مرة نوع جديد من الطفيليات وحيدة الجيل "بارانيللا ديبلودى" ينتمي ألي عائلة ميكركوتيليدي. أوضحت الدراسة التراكيب الداخلية وكذلك الخارجية وألقت الضوء على أهمية السطح الخارجي وأيضا دوره الوظيفي كعامل اساسي في تبادل الغازات وحماية الطفيل من المؤثرات البيئية المحيطة . كانت نسب الإصابة المسجلة بالأسماك قيد البحث كما يلي؛ اسماك البطيط ٤٨٪، أسماك القاصة ٢٨٪، أسماك الشعور ٢٢٪ وأسماك السيجان ١٦٪، علما بأنه قد تم فحص خمسين عينة من كل نوع من هذه الأسماك.